

insects in this disease. Samples were collected from diseased and dying trees during three surveys in 2007. Fungi and insects collected were identified based on morphology and in some cases using DNA sequence comparisons. Symptoms included a greying of the succulent branches, death of individual branches and eventually collapse of entire trees. Internal symptoms included browning and rotting of the tissues in the succulent branches, blue stain of the woody main stem and insect infestation. Several genera of fungi, including species of *Cibessii*, *Fusarium*, *Lasiodiplodia* and Ophiostomatoid fungi were isolated. Insects included several genera in the Curculionidae. Three different genera of Ophiostomatoid fungi were identified, of which two represent previously undescribed species. These fungi were also isolated from the scolytine beetles collected. *Lasiodiplodia theobromae* was the fungus most commonly isolated from the blue stained wood, and it was also common in the freshly developing brown lesions in the branches. Although the exact cause of the decline and death of the *E. ingens* trees is not yet clear, this study has provided a foundation for further investigations. Results also illustrate the great lack of knowledge regarding the fungal biodiversity on native tree species in South Africa.

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#### Symptomatic defence mechanisms include wound callose deposition after *Rhopalosiphum padi* L. infestation of barley leaves

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Wound callose deposition is a known rapid response of plants to wounding of its cells. The formation and deposition of wound callose during infestation of grain crops by phloem feeding aphids have been recently linked to the expression of visible damage symptoms by host plants. The deposition and subsequent symptoms expressed by infested plants were investigated during feeding on barley leaves by bird cherry-oat aphid (BCA), *Rhopalosiphum padi* L., an aphid that do not inflict any symptoms on host plants, especially at low level of feeding populations. Our results with aniline blue fluorochrome stain for callose observed using fluorescence microscopy, has revealed that wound callose deposition starts to appear after 14 days of feeding at low aphid levels (5 adult aphids) of infestation. Deposition of wound callose increases and becomes more pronounced with time to 21 days. Interestingly, feeding by larger populations (50 adult aphids), results in the formation and deposition of wound callose within 72 h, with deposition along longitudinal intermediate and cross veins. These results confirm that the formation and deposition of aphid-induced wound callose is partly involved in symptomatic expression of host plants during aphid feeding. Wounding responses include golden yellow streak symptoms that occur during heavy infestation by BCA.

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#### Molecular events in senescing soybean nodules

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Nodules play an important role as nitrogen fixating organs on leguminous plants such as soybean, enabling them to perform well even under nitrogen limiting conditions. The symbiosis has however a limited lifespan, and nitrogen fixation activity of the nodules usually ceases before the final pod filling stage. Pre-mature senescence of nodules caused by environmental stresses such as drought or cold can therefore affect the nitrogen metabolism of the plants and seed quality of the soybean crop. Nitrogen fixation capacity was monitored during different stages of soybean growth and RNA

transcripts in mature and senescing soybean nodules were compared. Understanding of the molecular processes in the senescing nodules can help to select key events responsible for the sudden decrease in nitrogen fixation capacity, and can lead finally to specification of new criteria for selection of soybean varieties for farming in unpredictable environments and nitrogen poor soils.

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#### Performance of seedlings subsequent to cryopreservation of embryonic axes of *Amaryllis belladonna*

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Cryopreservation is the most promising route for the long-term germplasm conservation of recalcitrant seeds. Since the ultimate aim of cryopreservation is the re-introduction of viable plants into the field, the vigour of plantlets derived from embryonic axes subjected to cryopreservation must be assessed. Embryonic axes excised from seeds of *Amaryllis belladonna* were subjected to one of the following treatment combinations: a) no cryoprotection, no dehydration and no freezing (fresh axes, *F*); b) cryoprotection with glycerol, rapid dehydration to  $\pm 0.20$  g/g and rapid rehydration (*D*); and c) cryoprotection with glycerol, rapid dehydration to  $\pm 0.24$  g/g, rapid cooling (hundreds of °C/s), rapid thawing and rehydration (*D*+*C*). Plants generated from these treatments were regenerated *in vitro*, hardened-off *ex vitro* and then exposed to either no water stress or a water deficit, imposed by withholding water (and then re-watered to see if they recovered). Embryonic axes subjected to partial dehydration (*D*) and the combination of partial dehydration and cooling (*D*+*C*) produced seedlings that were less vigorous than those from fresh axes. Failure to equilibrate with soil water potential overnight, indications of permanent leaf wilting, a decrease in potential photochemical efficiency and leaf chlorophyll content, as well as abnormal root growth characterised such seedlings. Seedlings derived from *D* axes performed better than *D*+*C* seedlings when water stressed, but seedling mortality was comparable in both treatments. Plantlets derived from cryopreserved *Amaryllis belladonna* axes may require special nursery treatment prior to re-introduction to the wild.

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#### Fields of papaya and honey: The scent of beetle-pollinated *Protea* species

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Inflorescences of four grassland and savanna *Protea* species in KwaZulu-Natal show features specialized to exploit generalist cetonid beetles. These include large open bowl-shaped inflorescences, extremely large pollen rewards, accessible dilute nectar, fruity/honey scent, colourful bracts, and a low growth form when frequently burnt. This contrasts to bird-pollinated *Protea* inflorescences that are typically robust, tall, unscented, with hidden nectar resources that are only accessible by a long beak or larger protea beetles. The scents of four *Protea* species were investigated using GCMS and the resulting chemical profiles analysed using correspondence analysis. The floral scents of *P. caffra*, *P. simplex*, and *P. dracomontana* resemble the fermenting fruit of *Carica papaya*, and share volatiles such as linalool, its oxides, and methyl benzoate. The scent chemistry of *P. welwitschii*, however, is highly complex with esters forming from a variety of acids and alcohols. Analyses involving dissected flowers revealed that the nectar is a main source of these odours, and that the scent production peaks with anthesis and nectar production. Scent preference tests with cetonid beetles confirmed that the odour of *P. simplex* is likely to be the principal attractant. Behavioural assays and GCEAD will be performed with these beetles and specific scent volatiles from the proteas in an attempt to explain the evolution of scent